

**FUNCTIONAL DIFFERENTIATION OF CHLORIDE CELLS
IN THE YOLK-SAC MEMBRANE
OF MOZAMBIQUE TILAPIA EMBRYOS**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Teleost fish maintain ion concentrations and osmolality of the body fluid at levels different from external environments. In adult fish, the gills, kidney and intestine are important osmoregulatory organs, creating ionic and osmotic gradients between the body fluid and external environments. In particular, gill chloride cells function as the salt-secreting site in seawater (SW) fish and probably as the ion-absorbing site in freshwater (FW) fish. In fish embryos and larvae, however, those osmoregulatory organs in adult fish are not yet developed or not fully functional. Nevertheless, embryos and larvae are also able to maintain ionic and osmotic gradients, and thus fish in early life stages should have other means of maintaining ion balance. In early life stages of fish when the gills are not yet developed, chloride cells are mainly distributed in the yolk-sac membrane, which covers the yolk, and the body surface (Kaneko et al., 2002). As the fish develop, the yolk is absorbed and, at the same time, the gills become functional. Thus, the functional site of chloride cells shifts from the yolk-sac membrane and body surface to the gills as the fish grow.

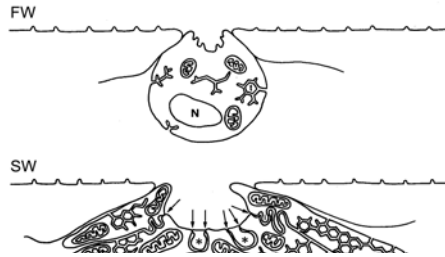


Figure 1. Freshwater (FW)- and seawater (SW)-type chloride cells in the yolk-sac membrane of tilapia embryos.

Occurrence of FW- and SW-type chloride cells

Mozambique tilapia *Oreochromis mossambicus* is a euryhaline teleost that is adaptable to a wide range of salinities from FW to SW. The tilapia can breed both in FW and in SW, and embryos and larvae can survive direct transfer from FW to SW, or *vice versa*. Taking advantage of the excellent euryhalinity of the tilapia, we examined morphological alteration of chloride cells in the yolk-sac membrane of the embryos and larvae in response to the environmental salinity.

In both FW and SW tilapia embryos and larvae, numerous chloride cells were detected in the yolk-sac membrane. However, chloride cells were much larger in SW fish than in FW fish. When FW-tilapia embryos were transferred to SW at the time of hatching, the chloride cell size increased significantly. Conversely, when SW embryos were transferred to FW at hatching, the chloride cell size decreased (Ayson et al., 1994). The enlarged chloride cells in SW formed multicellular complexes together with adjacent accessory cells, whereas chloride cells existed individually in FW (Figure 1; Shiraishi et al., 1997). Immunocytochemical studies showed that the CFTR Cl⁻-channel and Na⁺, K⁺, 2Cl⁻-cotransporter were located in the apical and basolateral membranes, respectively, in the SW-type chloride cell complexes, but not in the FW-type cells. Moreover, the chloride test and X-ray microanalysis revealed that the SW-type complexes had definitive function of Cl⁻ secretion. These findings clearly indicate a significant role of chloride cells in the yolk-sac membrane in adaptation to SW.

Functional differentiation of chloride cells

To examine the development of SW chloride cells, we observed *in vivo* sequential changes in chloride cell morphology during SW adaptation using a confocal laser scanning microscope (Hiroi et al., 1999). When transferred from FW to SW, single (FW-type) chloride cells enlarged and were indented by newly-differentiated accessory cells to form multicellular complexes (SW-type), suggesting plasticity in the ion-transporting functions of chloride cells. To further examine the functional differentiation of chloride cells, we developed a “yolk-ball” incubation system, in which the yolk sac was separated from the embryonic body and subjected to *in vitro* incubation (Shiraishi et al., 2001). The incision on the yolk ball was healed during incubation in balanced salt solution for 3 h, so that the yolk-sac membrane completely enclosed the yolk. The yolk balls prepared from FW-acclimated embryos were transferred either to FW or to SW, and incubated for up to 96 h. In the yolk balls transferred to SW, chloride cells often formed multicellular complexes, characteristic of SW-type chloride cells. In those transferred to FW, on the other hand, the cells were small and rarely formed a complex. The Cl⁻-turnover rate measured by the whole-body influx of ³⁶Cl⁻ was about 60 times higher in the yolk balls in SW than in FW. Such responses of the yolk balls were identical to those observed in intact embryos.

These findings indicate that the chloride cells in the yolk-sac membrane are equipped with an autonomous mechanism of functional differentiation, independent of embryonic endocrine and nerve systems. The yolk-ball incubation system established here definitely serves as an excellent experimental model for further studies on chloride cell differentiation and functions.

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